

Influence of Light Quality and Intensity on Adventitious Root Formation in Microshoots of *Pinus pinea* L.

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Abstract

In the present study of *Pinus pinea* L., further improvement of microshoot rooting was achieved by applying Cool-white light at increased intensity from 60 to 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In contrast, light provided by Gro-lux lamps promoted rooting of the microshoots at the same frequency regardless of its intensity. Majority of microshoots (70.4%) grown under Cool-white lamps at the intensity of 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were also significantly taller when compared with those from other tested treatments.

INTRODUCTION

The light-mediated changes in plant growth and development are referred to as photomorphogenesis, and plants have extraordinary versatility to perceive different light signals in different developmental contexts. Different groups of photoreceptors of the photosensory systems regulate plant development, namely cryptochromes, phototropins and phytochromes each of them monitoring different wavelengths regions of the spectrum (Quail, 2002). Plants use phytochrome to detect and respond to red and far-red wavelengths and cryptochromes were the first blue light receptors isolated and characterized. Light can also modify the efficacy of plant growth regulators (PGRs) as well as affect the endogenous hormone balance. Auxin plays a central role in the determination of rooting capacity, and light conditions are known to affect auxin metabolism and tissue receptivity (Reid et al., 1991). From an applied point of view, plant morphogenesis may be influenced by the correct choice of lamps and filters (Fuerakranz et al., 1990). For example, red-light improved rooting percentage and root numbers in shoots of two genotypes of grape propagated in vitro (Poudel et al., 2008). However, little is known about the effect of green and yellow lights, which seem to be involved in the regulation of in vitro plant development (Loreti et al., 1991).

The purpose of this work was to establish if light quality and intensity, within the visible range, influence root growth and development of *Pinus pinea* microshoots with the aim of enhancing the present rooting protocol achieved with Cool-white (CW) lamps.

MATERIAL AND METHODS

Plant Material

Cotyledons from non-germinated embryos of stone pine (*Pinus pinea* L.) were used as explants. The seed coat was cracked with a nut cracker and discarded. The remainder megagametophytes were surface sterilized by immersion in 70% (v/v) ethyl alcohol for 2 minutes followed by three rinses in sterile bi-distilled water. They were then disinfected with sodium hypochlorite 10% (v/v) (commercial bleach with 5% free chlorine) for 25 minutes followed by four rinses in sterile bi-distilled water. All of the following steps were carried out under aseptic conditions. An embryo was excised from the megagametophyte by making a longitudinal incision with a scalpel and by gently pulling the edges of the cleft with two forceps. Finally, the cotyledons were excised from

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the embryo axes with a cut at their bases.

Microshoot Induction from Embryo Cotyledons

The whole set of cotyledons of each of 20 excised embryos was cultured separately in a Petri-dish (9 x 1.5 cm) containing WPM (McCown and Lloyd, 1981) medium supplemented with 5 mg/L of benzylamino purine (BAP) for shoot organogenesis. After a month, the explants with shoot buds were transferred to a fresh PGR-free medium with 2 g/L of activated charcoal (AC) to promote shoot elongation.

Rooting of Microshoots

Seven, eleven, and six clonal shoots were tested at 60, 90, and 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. In all experiments, shoots 2 cm in height were transferred to WPM with half concentration of the macronutrients, 0.65% Difco Bacto-Agar and different carbon sources (see below), and adjusted to pH 5.8 before autoclaving. For root induction, which lasted two weeks, the medium was supplemented with 10.7 μM naphthalene acetic acid (NAA) (Oliveira et al., 2003), and 0.12 M glucose (WPMRI). The cultures were kept for two weeks in a growth chamber; the first week in darkness. During the 2nd week of induction (under 16 h photoperiod and constant temperature of 19°C) and during root expression phase, two light sources were used: Sylvania Gro-lux lamps 18W (GL) and Philips Cool-white lamps 18W (CW) at different photosynthetic photon flux (PPF – see above) depending on the experiment. In all experiments root expression medium was WPM consisting of half concentration of the macronutrients, without PGRs and with 0.058 M glucose (WPMRE) at 16 h photoperiod and 24/19°C day/night temperatures. The root emergence was monitored for six weeks.

Light Sources Spectra

Gro-lux: The color tone of GL light is violet; this is the result of the combination of blue and red wave lengths. Cool-white: Broad-spectrum CW light lamps supply blue, yellow, and green light but very little far red light (Fig. 1). Light intensity was measured in the middle of the culture flask with the quantum sensor (Skye Instruments Ltd., UK SKP 200).

Evaluated Parameters and Statistical Analyses

Rooting percentages and the number of roots produced over time for each light quality and intensity were monitored. The percentages of rooted microshoots were compared by the analysis of variance (ANOVA) for clones and light sources using Statistica six Sigma. Means were compared by Duncan's Range test.

RESULTS

P. pinea microshoots rooted at 40% under CW lamps and at 47.1% under GL lamps at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 2, Experiment 1). In order to test if increased to 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity from both types of lamps would influence the rooting response; one additional lamp was added in each tissue culture chamber (Experiment 2). Since there were no statistical differences among clones in the first experiment, the data analysis in experiment 2 considered only light quality and light intensity. In the latter experiment 70.4% microshoots rooted under CW and 51% under GL lamps (Fig. 2). There was a significant difference in the rooting percentage under CW lamps at 60 versus 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$, but no difference was observed at the two intensities for GL lamps (Fig. 2, Table 1). This indicated that the *P. pinea* microshoots were not sensitive to variation in the photosynthetic photon flux (PPF) from GL lamps with respect to rooting response. When the light was increased to 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$, reduction of the rooting percentages was observed for both lamp types, particularly for GL (Fig. 2).

The number of roots produced per week increased exponentially during two weeks on WPMRE medium under both types of light (Fig. 3).

DISCUSSION

During the first week of the rizhogenic process (induction) the combination of 0.12 M glucose with 10.7 μ M NAA in the medium, and continuous darkness were the most favorable conditions compared with previously tested ones (unpublished results).

Rooting percentage was influenced by different light quality according to the PPF used. The fact that similar rooting percentages were obtained for GL lamps at 60 and 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$ indicated that an increase in light intensity did not stimulate rooting. In contrast, increased intensity of CW light increased the rooting percentage by more than 30%. This is an interesting result from the practical point of view because without any change in the tissue culture protocol, except for one additional CW lamp in the culture chamber, it was possible to obtain an increased number of rooted stone pine plantlets. However, at a higher light intensity (110 $\mu\text{mol m}^{-2} \text{s}^{-1}$) ARF was inhibited under both lamps indicating that optimal conditions were exceeded.

Comparing both fluorescent lamps spectra, GL lamps emit blue and red waves and CW more yellow and green. These differences in the spectra could explain the results of this study, which are consistent with the previous observations that phytochrome was mainly involved in root formation (Tyburski and Tretyn, 1999) and that blue light inhibited photomorphogenesis (Seibert et al., 1975). The yellow component of CW lamps might have enhanced adventitious rooting in *P. pinea* similarly to the results of Fuerakranz et al. (1990). The authors reported that yellow light and CW lamps were superior for rooting of *Prunus serotina* compared with red and blue lights alone.

Maximum rooting that occurred after approximately 15 days in the expression medium and was consistent in all the treatments, indicated that the time to trigger microshoot response and growth of new roots were independent from the light quality, and that this parameter was determined by other physiological factors.

ACKNOWLEDGEMENT

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Table

Table 1. Duncan test for variable rooting percentage.

	Light quality	Intensity	GL 60, 47143	GL 90, 45714	GL 110, 05000	CW 60, 42643	CW 90, 71571	CW 110, 16667
1	GL	60		0,8900	0,0005*	0,6830	0,0229*	0,0089*
2	GL	90			0,0007*	0,7663	0,0215*	0,0103*
3	GL	110				0,0012*	0,0000*	0,2629
4	CW	60					0,0127*	0,0161*
5	CW	90						0,0000*
6	CW	110						

Figures

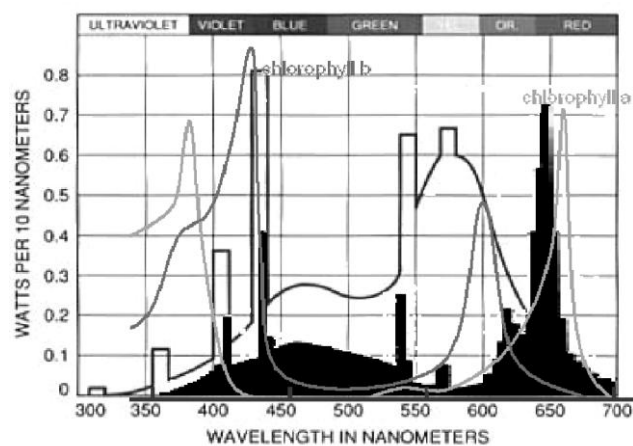


Fig. 1. Spectra of Sylvania Gro-lux lamps (white) and Philips Cool-white lamps (black).

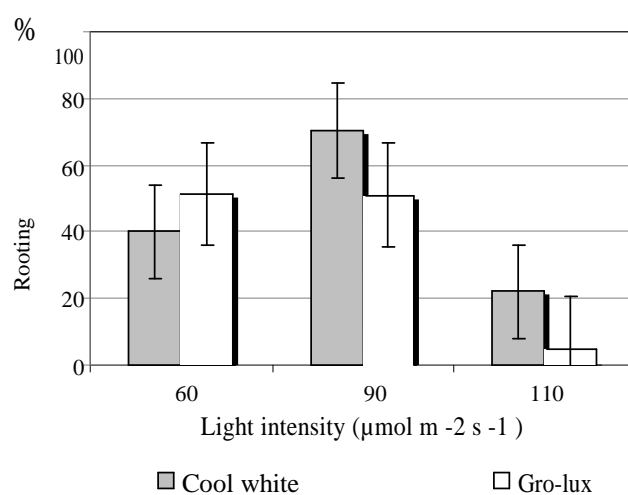


Fig. 2. Rooting of *P. pinea* microshoots under different light quality and intensity. See significance in the Duncan Range test above.

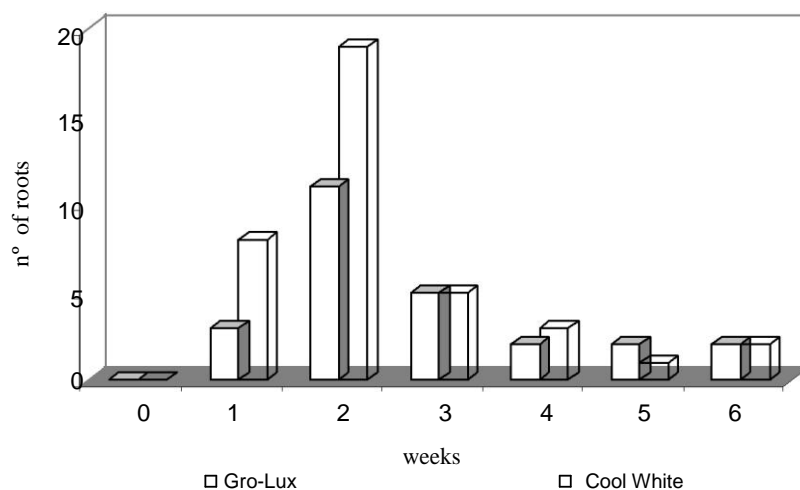


Fig. 3. Number of roots produced per week considering all microshoots for each light treatment under Cool-white and Gro-lux lamps at 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$.